Supporting information

Spin Delocalization Over Type Zero Copper Site

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Assignment of the 14N signals of C112D

$A_1(^{14}N)$=27.9 MHz and $A_2(^{14}N)$=36.9 MHz give $A_1(^{15}N)$ =39.1 MHz and $A_2(^{15}N)$ =51 MHz, respectively. This should give ^{15}N lines at 5.3 and 33.8 MHz and 11.6 and 40.1 MHz, respectively. The ED-NMR spectrum recorded at $g_\perp$, shown in Fig. S1a, exhibits peaks ~12 MHz and 33.8 MHz (marked with arrows) and the ENDOR spectrum, Fig. S1b, shows a broad signal in 40-44 MHz. This is in agreement with the above expectations. We exclude the possibility that the peak at 33.8 MHz in the ED-NMR spectrum is due to weakly coupled ^{13}C because it is not at the correct frequency (compare with Fig. S1b). Furthermore, the ^{13}C signals are expected to be very weak in the ED-NMR because the ^{13}C hyperfine coupling is far from the cancelation condition. The alternative of one type of nitrogens with $A(^{14}N)$=32.4 MHz would yield ^{15}N signals at 8.5 and 36.9 MHz which are not observed. The S/N of the ^{15}N ED-NMR spectrum is much lower than that of the corresponding ^{14}N spectrum because of the forbidden transition are less intense due to the absence of the nuclear quadrupole interaction that contribute significantly to the mixing of the nuclear states.

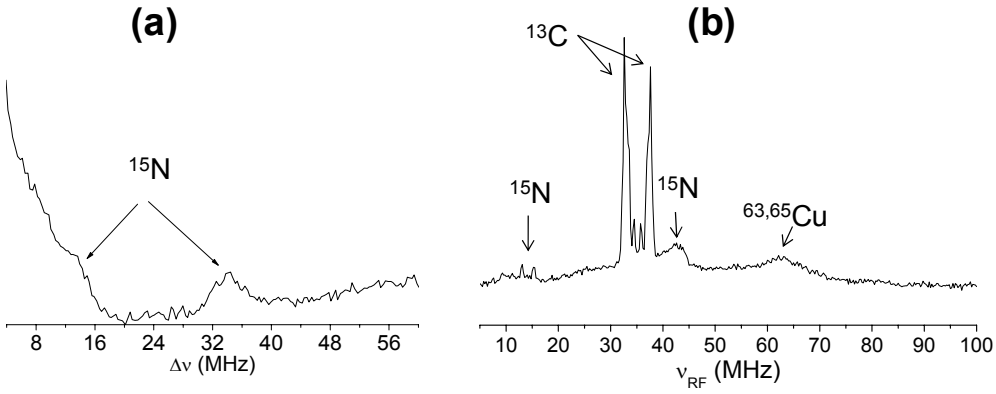

Figure S1: (a) W-band ED-NMR and (b) Davies ENDOR of ^{13}C/^{15}N enriched C112D measured at 3289 mT. The arrows mark the observed peaks and their assignment.

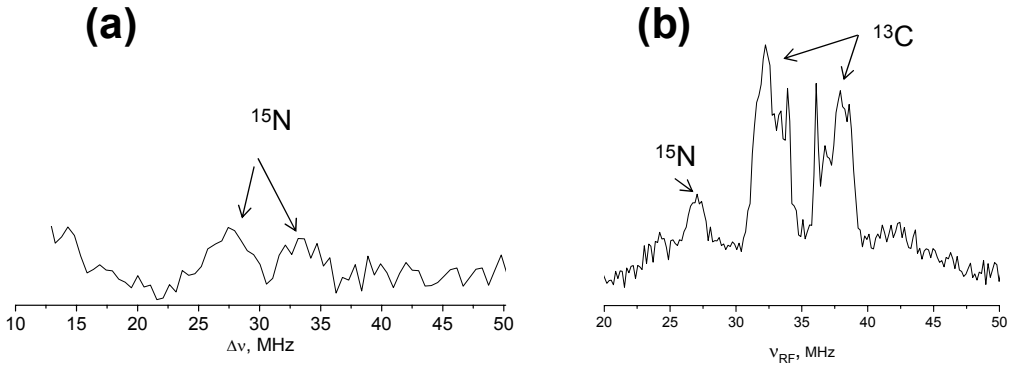

Figure S2. ED-NMR (a) and Davies ENDOR (b) of ^{13}C/ ^{15}N enrich C112D/M121L recorded at B=3267 mT. The broad background in (b) is attributed to ^{63,65}Cu signals.

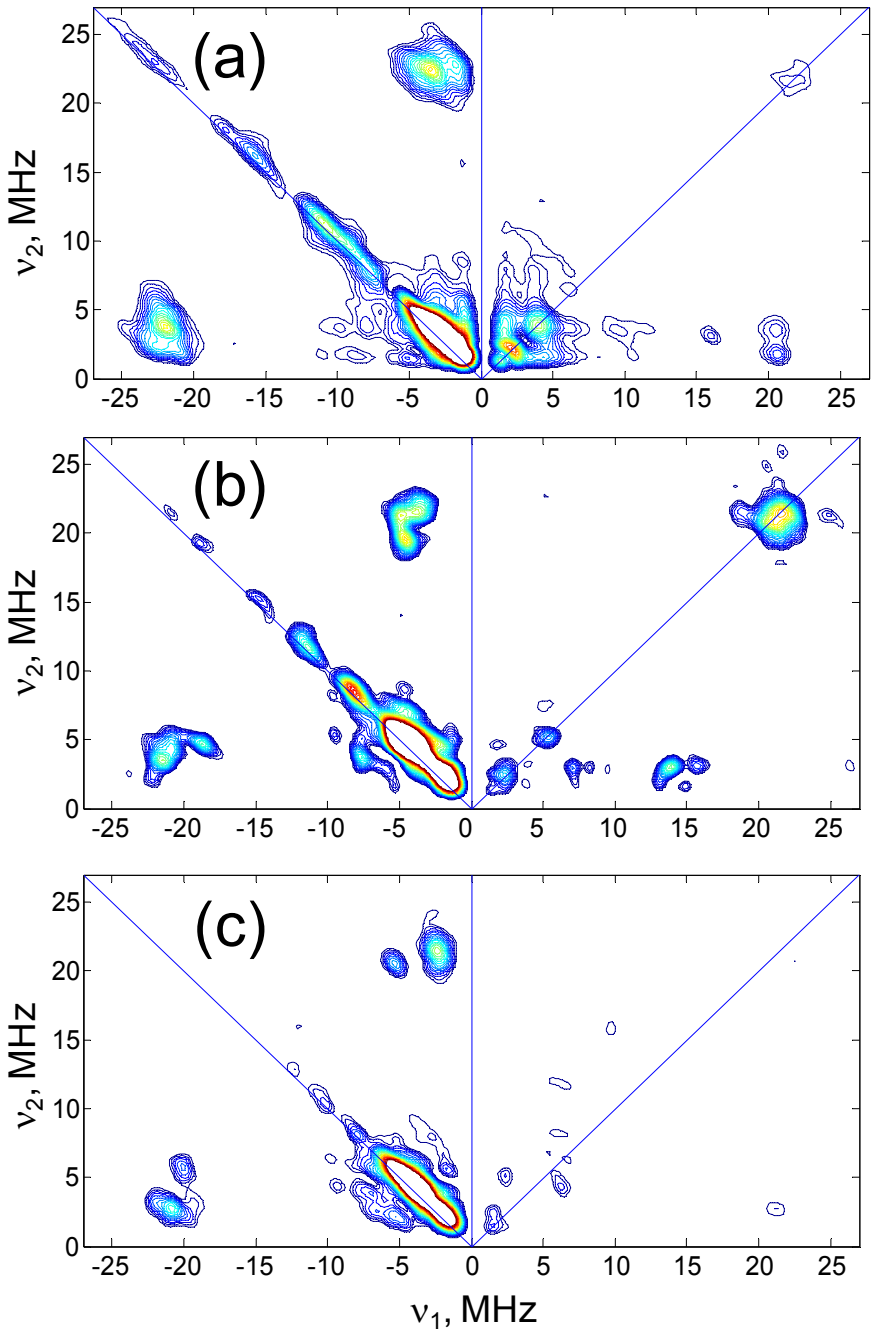

Figure S3. W-band HYSCORE spectra of C112D/M121L recorded at B=3267 mT recorded with τ=137.5 ns and B_0=3267 mT (a), and at B_0=2881 mT and τ=137.5 ns (b) and τ=200 ns (c).

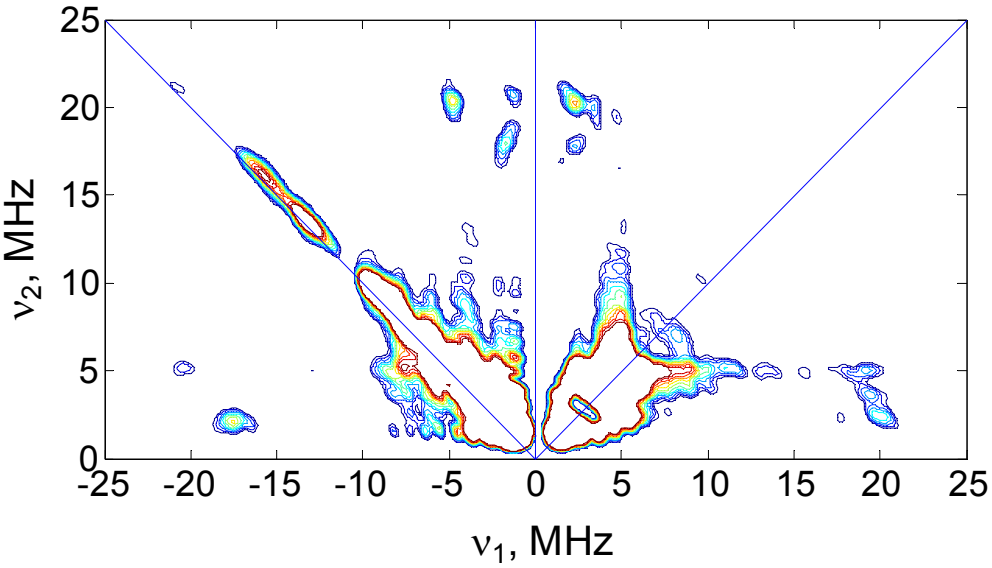

Figure. S4: W-band HYSCORE spectra of C112D/M121F azurin recorded at B=3242 mT and τ=150 ns.

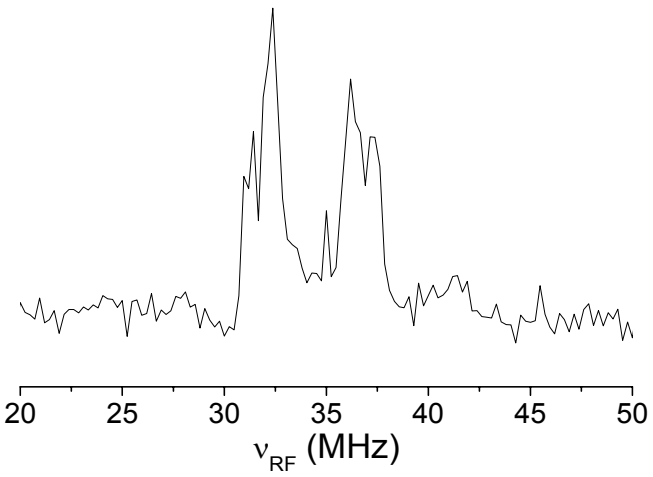

Figure S5. W-band Davies ENDOR of $^{13}C/^{15}N$ enriched C112D measured at 3222 mT.

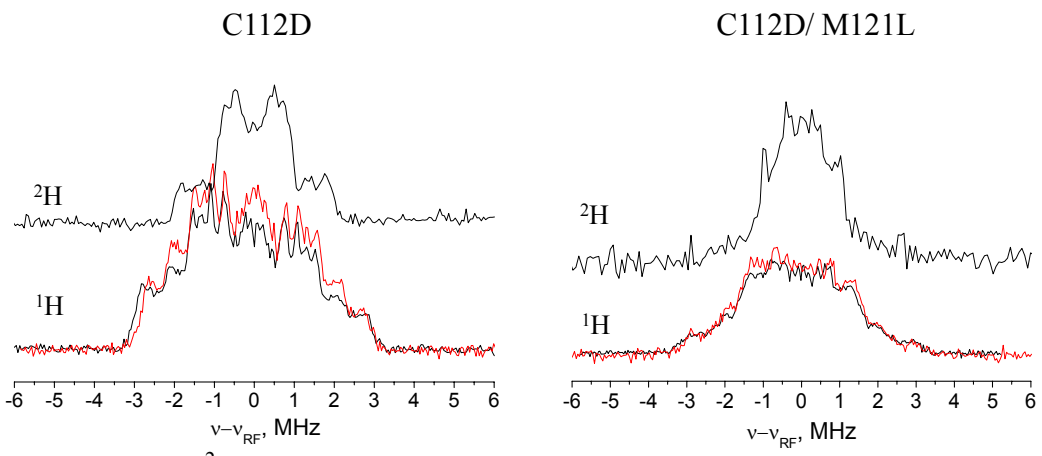

Figure. S6 Top: ^{2}H Mims ENDOR spectra of C112D and C112D/M121L in D_2O solutions (scale was multiplied by $\gamma(^1H)/\gamma(^2H)$). Bottom: ^{1}H Davies ENDOR spectra of these mutants in H_2O (red trace) and D_2O (solid trace). The spectra were acquired at the magnetic field where echo intensity is the largest.

Table S1 The assignment and frequencies (MHz) observed in the X-band HYSCORE spectra of the mutant studied. Here we use in parenthesis the NQR (nuclear quadrupole resonance) notations. At exact cancelation the nuclear frequencies in the α-manifold are the same as the NQR frequencies (ν_0, ν_-, ν_+)

	C112D/M121F		C112D/M121L		C112D	
	$g_\perp$	$g_\parallel$	$g_\perp$	$g_\parallel$	$g_\perp$	$g_\parallel$
ν^β_{dq}, $\nu^\alpha_{sq1,2}$ ($\nu_{0,-}$) (a)	4.2,0.7	4.0,0.6	4.3,0.7		4.1,0.7	4.0,0.8
ν^β_{dq}, $\nu^\alpha_{sq1,2}$ ($\nu_{0,-}$) (b)	3.4,0.8	3.6,0.6	3.5,0.8	3.8,0.7	3.7,0.7	3.7,1.0
ν^β_{dq}, ν^α_{dq} (ν_+) (a)	4.2,1.5	4.0,1.5	4.3,1.5	4.1, 1.7	4.1,1.5 3.7,1.6	4.0,1.7
ν^β_{dq}, ν^α_{dq} (ν_+) (b)	3.6,1.5	3.6,1.5	3.5,1.6	3.7,1.7		3.7,1.8
(ν^β_{dq}, $\nu^\alpha_{dq}+\nu^\alpha_{sq1,2}$ ($\nu_{0,-}+\nu_+$) (a)		4.0,2.2				
(ν^β_{dq}, $\nu^\alpha_{dq}+\nu^\alpha_{sq1,2}$ ($\nu_{0,-}+\nu_+$) (b)		3.6,2.2				

Table S2. Summary of the Cu-H distances obtained from the crystal structures (C112D: 3FQY; C112D/M121L: 3FPY) and the corresponding dipolar coupling $T_{\perp}=1/2T_{\parallel}$.

double mutant C112D/ M121L	r, Å	$T_{\perp}$,, MHz	single mutant C112D	r, Å	$T_{\perp}$, MHz
His46					
(non-exchangable)					
α	2.61	4.33		2.34	6.01
ε1	2.93	3.06		3.26	2.22
β	3.78	1.43		3.53	1.75
β	4.63	0.78		4.50	0.84
(exchangable)					
backbone amide	4.65	0.77		4.83	0.68
ε2	4.85	0.67		5.05	0.60
His117					
(non-exchangable)					
ε1	3.23	2.28		3.29	2.16
β	2.98	2.91		3.02	2.80
β	2.73	3.78		3.37	2.01
(exchangable)					
backbone amide	5.99	0.36		4.83	0.68
ε2	5.05	0.60		5.06	0.59
Phe114					
(non-exchangable)					
β	2.99	2.88		2.91	3.12
β	3.84	1.36		3.71	1.51
(exchangable)					
backbone amide	4.58	0.80		4.16	1.07
Leu121			Met121		
(non-exchangable)					
δ1	2.81	3.47	ε	3.49	1.81
δ1	4.06	1.15	ε	3.92	1.28
δ1	4.26	1.00	ε	4.92	0.65
Asn47					
(exchangable)					
backbone amide	6.16	0.33		4.04	1.17